THE INHERITANCE OF RUST IMMUNITY OCCURRING IN *LINUM ANGUSTIFOLIUM* (HUDS.)

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ABSTRACT

The inheritance of immunity to *Melampsora lini* attacking cultivated flax, was studied in two introduced lines of *Linum angustifolium*, 8-2 and 13-4.

The F_1 hybrids between rust susceptible varieties of *L. usitatissimum* and rust-immune lines of *L. angustifolium* were immune to races 179 and 258 of *M. lini*. This indicated that the immunity to rust in both these lines of *L. angustifolium* was conditioned by dominant genes. The results of reciprocal crosses revealed that there was no maternally inherited influence upon rust immunity in either of these lines.

Line 8-2 of *L. angustifolium* was found to carry two dominant genes conditioning immunity to rust. These genes were designated as L_A and X by the author. Gene L_A was found to belong to the L allelic series of rust resistant genes in *L. usitatissimum* and conditioned immunity to race 179 but not to race 258. Gene X conditioned immunity to race 258 but not to race 179. These two genes were linked, having a crossover distance of 5.1 ± 1.0 units between them.

Line 13-4 was found to carry two genes conditioning immunity to *M. lini*. One of these genes was completely dominant and conditioned immunity to races 179 and 258. The other gene appeared to be incompletely dominant and able to condition immunity to these two races when in the homozygous state only.

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF ILLUSTRATIONS

INTRODUCTION

The disease, rust of flax, caused by the fungus Melampsora lini (Pers.) Lev. exists in many pathogenic forms. The large number of these different pathogenic races indicates the wide potential virulence of the pathogen. The most successful means of combating this disease has been by the use of rust-resistant varieties; thus it is desirable to have available many different sources of host resistance which can be incorporated by breeding methods into cultivated varieties.

Dr. A. W. Henry of the Division of Plant Pathology at the University of Alberta, found two introductions of the wild flax Linum angustifolium Huds. which had immunity to M. lini. The purpose of this study was to determine the nature of inheritance of this rust immunity, the number of genes involved, their mode of action and the possibility of their being associated with any of the allelic series of rust resistant genes previously discovered in L. usitatissimum L. .

LITERATURE REVIEW

Differential Pathogenicity in Flax Rust

M. lini is an eu-autoecious, heterothallic obligate parasite of flax as reported by Arthur (3) and Allen (2). According to Buchheim, as reviewed by Flor (6), Kornicke in 1865, was the first to suggest the occurrence of physiologic specialization in this rust. This was based upon the rust's ability to attack some species of the genus *Linum* but not others. Evidence of a similar nature was reported by Arthur (3), Hart (16) and Henry (18).

Flor (6) distinguished fourteen pathogenic forms of *M. lini* on the basis of their reactions to nine different varieties of cultivated flax, *L. usitatissimum*. Similar investigation which followed, added to the number of pathogenic forms known (7, 26).

Flor (9, 11) studied the genetics of virulence in the pathogen. He found that virulence of rust upon varieties of cultivated flax was conditioned by recessive genes, with the exception of virulence upon the flax variety Williston Brown. On this variety a dominant gene in the fungus conditioned virulence.

The Genetics of Resistance to Flax Rust

Biffen (4) was the first to demonstrate that disease resistance in plants was inherited according to Mendelian laws. Henry (19) showed that immunity to a mixture of North American races of rust was conditioned by the dominant allele of a single pair of genes in the flax varieties Ottawa 770B and Bombay, and by the dominant allele of either of two pairs of genes in an Argentine selection of flax.

As investigation proceeded into the inheritance of resistance to rust in flax, it became apparent that numerous genes may condition resistance and that these genes occur in several allelic series or groups of closely linked genes. Myers (23) studied the inheritance of resistance to several North American races of rust in seventeen varieties of flax. From this investigation he concluded that rust resistance was conditioned by duplicate genes in two allelic series; these he designated as the L and M series. Myers found that immunity was dominant and epistatic to susceptibility, near immunity and near resistance; near immunity was hypostatic to immunity; and near resistance was hypostatic both to near immunity and immunity. The occurrence of rust resistant genes of the host in allelic series was further investigated by Flor (8, 9, 12, 14). He reported the existence of twenty-five gene pairs conditioning resistance to rust in the flax host. These were located in five groups, three of which were independently inherited allelic series while the other two were series between which some linkage had been demonstrated.

Mayo's (22) critical examination of Flor's work indicated that Flor's evidence for allelism established much less than was claimed. This was said to be so because the work of Flor was based largely on the classification of the F_2 progenies from F_1 heterozygotes in the repulsion phase. Mayo suggested that in future work more critical tests should be carried out by using progeny of heterozygotes backcrossed to a universally susceptible variety.

The majority of rust resistant genes in flax were completely dominant in their action (12, 19, 22). However, Flor (8) reported

the occurrence of a gene in the variety Buda which was incompletely dominant in conditioning resistance to rust. Plants heterozygous for this gene were distinctly less resistant to some races of rust than plants which were in the homozygous dominant condition.

From the study of the genetics of rust resistance in the flax host (12) and the genetics of virulence in the pathogen (11), Flor has postulated the existence of a gene-for-gene relationship between the genes of the host, which condition resistance, and the genes in the pathogen conditioning virulence (12, 13, 14, 15). The specificity of this relationship suggested that the conditions necessary for the pathogenic reaction were much more precise than those necessary for resistance. A pathogen carrying a single gene conditioning virulence was able to parasitize only a host which carried no genes for rust resistance or else only the one specific gene for resistance against which the pathogenic gene had been selected to act (14, 15). This implied that a single gene conditioning rust resistance in the host may be of considerable importance in conditioning resistance to a number of genes for virulence carried in various races of the pathogen. The actual occurrence of such a gene was demonstrated in the rust immune variety of flax Ottawa 770B (17). The single gene in this variety conditioned resistance to all North American races of *M. lini*.

Classification of the Rust Reaction in Flax

A number of systems have been used to classify the flax host's reaction to rust. Henry (19) classified host plants which

showed no sign of rust infection as immune and those on which a pustule appeared as susceptible.

Flor's (6) system of classification differed from Henry's in that the former recognized the following classes: near immune, resistant, semi-resistant, moderately susceptible and highly susceptible. This system was similar to that used by Stakman and Levine (24) to classify wheat reaction to Puccinia graminis Pers. Myers (23) extended Flor's original system to include eleven different classes of infection types.

Flor (6, 7) noted that rigid rules for pustule classification could not be followed as rust reaction was variable and dependent upon such factors as light intensity, length of day, temperature, and the age and vigor of the host plant. Flor (10, 11, 12) later adopted a trichotomous system of classification in which only two types of non-susceptible reactions, immune and resistant, were recognized. This system was subsequently modified to a dichotomous system (13) in which only two reactions, resistant and susceptible, were recognized. The new resistant class consisted of those plants previously classified as immune and resistant. It was found that varieties classed as semi-resistant in the greenhouse tests were resistant in the field tests.

Deshpande and Jeswani (5) as well as the author have preferred to follow the classification used by Henry (19) in which all plants exhibiting a rust reaction, whether slight, medium or heavy, were classed as susceptible.

Rust-Reaction of Wild Species of Linum

Henry (18) demonstrated that immunity to M. lini occurred in the species Linum perenne L., L. austriacum L., L. grandiflorum Desf., L. flavum L., L. catharticum L., and certain strains of L. usitatissimum L. and L. angustifolium Huds.

Tammes (25) demonstrated that L. angustifolium Huds. and L. usitatissimum L. were readily crossed and produced fertile hybrid offspring.

Deshpande and Jeswani (5) studied the inheritance of rust resistance in two wild species of flax, L. africanum and L. angustifolium. These species were crossed to a susceptible variety of L. usitatissimum. The F_1 progenies were immune to M. lini indicating complete dominance of immunity to susceptibility. The F_2 segregates were classified as immune or susceptible according to Henry's (19) system of classification. Their results demonstrated that L. africanum contained one gene conditioning rust immunity. These authors were unable to explain the inheritance of the rust resistance found in L. angustifolium.

MATERIALS AND METHODS

Two lines of L. angustifolium, which were immune to a local collection of M. lini, were discovered by Dr. A. W. Henry, Division of Plant Pathology at the University of Alberta. He designated these as lines 8 and 13. Line 8 was introduced from Switzerland in 1952 and line 13, from Germany in 1956. Single plant selections were made from each of these two lines by Dr. Henry and the progenies of two of these selections, 8-2 and 13-4, were used in this study. These selections are henceforth referred to as 8-2 and 13-4 throughout the remainder of this paper.

The varieties of L. usitatissimum with which these lines of L. angustifolium had been crossed were: Bison, Redwing, Dakota, Stewart and Akmolinsk. The varieties Bison and Redwing were obtained from the Department of Plant Science, University of Alberta. The varieties Dakota, Stewart and Akmolinsk, were taken from samples of 18 host tester differential varieties obtained from Dr. H. H. Flor, Pathologist, State College Station, Fargo, North Dakota. These 18 differential varieties were used to identify the races of M. lini used in this investigation.

Two races of M. lini, 179 and 258, were used to study the rust reaction of the experimental material. Race 179 was obtained from an urediospore collection several races obtained from Dr. B. Peturson, Plant Pathologist at the Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba. This sample of rust was propagated on the flax varieties Williston Brown and Bison. It was frequently checked for

its racial purity by passing it through Flor's host tester differential varieties and was found to be consistently virulent upon the varieties Dakota, Williston Brown and Bison; and avirulent in its reaction to the other 15 varieties of the differential set.

Race 258 was obtained as an urediospore sample from Dr. H. H. Flor. This was propagated on the flax varieties Stewart and Bison and was also frequently checked for its purity on Flor's host tester differential varieties. In all cases this culture of race 258 showed virulence upon the varieties Dakota, Stewart, Koto, Williston Brown, Victory A and Bison; and avirulence on the remaining 12 varieties of the differential set.

Each of the two selections of L. angustifolium was crossed to two rust susceptible varieties of L. usitatissimum, Redwing and Bison. The variety Bison was used as a susceptible parent as it had been reported to be susceptible to all North American races of M. lini (12, 14). Redwing was used as a susceptible parent because of the agronomic value it has in the area in which the investigation was carried out.

Line 8-2 was reciprocally crossed with three tester varieties of L. usitatissimum, each carrying a single gene for rust resistance in a different allelic series. The varieties used were Dakota of the M series, Stewart of the L series and Akmolinsk of the P series. The F_1 hybrids of these crosses were testcrossed to Bison. This was done to test for allelism or close linkage between the genes for immunity in 8-2 and the genes for immunity in the tester

varieties of L. usitatissimum. A sufficient number of testcrosses were made to the F_1 hybrids to insure the detection of linkage having a p value > 0.01 (1).

The F_1 hybrids between 13-4 and Bison were backcrossed to Bison.

All the plants tested for their rust reactions were inoculated while in the seedling stage when they were four to six inches high. Where excised shoots were used they were cut from the seedling when the portion of the seedling above the cotyledon was approximately three inches high.

The urediospores of M. lini were applied either in the pure form or diluted with talc, depending upon the quantity available at the time of inoculation. The maximum dilution used was 25 parts of talc to one part urediospores. Where there were only a few plants to be inoculated and there was an abundant supply of urediospores, the spores were applied directly to the terminal bud of the seedling with the aid of a small camel's hair brush. Where numerous seedlings or excised shoots were inoculated, the spores were diluted with talc and applied with a small powder blower.

Prior to inoculation all the seedlings were sprayed with tap water. After the spores had been applied, the seedlings were again sprayed with tap water, this time very lightly; then they were immediately transferred to an incubator, where they were kept at 65° C. and a relative humidity of 100% for 48 hours. After incubation the seedlings were returned to the greenhouse bench.

Seedlings which were grown directly in the greenhouse

bench were incubated following inoculation by covering the entire bench with polyethylene sheeting supported on wire frames. This covering maintained the relative humidity at 100% during the 48 hour incubation period. The temperature of the compartment in which the bench of seedlings was located was maintained at a mean temperature of 65° C.

Individual F_1 plants from all crosses were grown in the greenhouse in 4½ inch pots. They were tested for their rust reactions at the seedling stage by inoculating them with urediospores of a race of M. lini to which the L. usitatissimum parent was susceptible. After incubation the seedlings were placed on the greenhouse bench. The rust reaction of each seedling was recorded twelve days after inoculation.

The F_2 seedlings of the hybrid 13-4 x Bison and its reciprocal were grown in flats for rust classification. These flats were filled with a 3:1:1 mixture of soil, sand and peat. Both the flats and the soil mixture were sterilized prior to seeding. Each flat contained 198 seedlings planted in 18 rows spaced one inch apart. The plants within each row were also spaced one inch apart. Each flat contained one row of the parent varieties plus a seedling of each of Flor's differential varieties.

The seedlings were inoculated with an urediospore-talc mixture of race 179. Following incubation the seedlings were placed on the greenhouse bench and 14 days after inoculation the rust reaction of each seedling was recorded.

The F_2 seed of some of the hybrids of 8-2 and 13-4 crossed

with Bison and Redwing were sown directly in the greenhouse bench. The seeds were sown one inch apart within the rows and nine inches apart between the rows. Check rows of each parent were included. These seedlings were inoculated with a urediospore-talc mixture of race 258. Incubation following inoculation was carried out on the greenhouse bench by covering the bench with polyethylene sheeting. The rust reaction of these seedlings was recorded 12 days after inoculation.

The B_1(1) and T_1(2) progenies obtained from crossing F_1 hybrids to the variety Bison were tested for their rust reaction by the use of Kerr's excised shoot technique (20). This technique allowed for the testing of a single plant with several different races of rust without fear of either contaminating the races one with another, or losing the plant through susceptibility.

Five trays, to hold the excised shoots, were made from $\frac{1}{4}$ inch thick "Perspex" plastic. Each tray was 14 inches x 9 inches in dimensions and fitted, as a lid, over a Pyrex cake dish of the dimensions 14 x 9 x 2 inches. Holes, 1/8 inch in diameter, were drilled in each tray. The first tray made had 204 holes drilled in it, spaced in a grid 3/4 of an inch apart (Figure 1). The capacity of subsequent trays was increased in that each had 272 holes drilled in it, spaced in a grid 7/10 of an inch apart. The trays, and the holes within each tray, were numbered.

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(1) First generation backcross

(2) First generation testcross

Figure 1. Tray and dish to hold excised shoots.

The seedlings to be excised were grown in a 3:1:1 mixture of soil, sand and peat in 20 x 12 x 4 inch flats. Both the flats and the soil mixture were sterilized prior to seeding. The seeds were sown in these flats spaced in a one inch grid, with 17 rows in each flat and 11 plants in each row. Check plants of the parents, plus at least one set of the 18 varieties of Flor's host tester differentials, were included in each group of seedlings which were to be excised together and placed in the same tray for rust inoculation. These check plants tested the completeness of inoculation and the purity of the race of rust being used.

When the seedlings were approximately four inches high the portion of the stem above the cotyledon was excised. Each excised

shoot thus obtained was placed in one of the holes of the excised shoot tray. The Pyrex dishes under each tray were full of tap water and the water level of each was maintained at the point of overflow by a continuous supply of water from an adjacent crock. The flow of water from each crock into its corresponding Pyrex dish was regulated by a screw type hose clamp on a rubber delivery tube. The high water level in each dish was necessary to insure that the base of each shoot was always immersed.

After the shoots from all the seedlings had been excised and placed in the trays, they were taken to an isolated room and inoculated with a spore-talc mixture of one of the races of M. lini used in the test. The shoots were then incubated and twelve days from the time of inoculation the rust reaction of each shoot was recorded. The rust reaction of each shoot was indicative of the rust reaction of the seedling from which it was excised.

The trays containing the inoculated excised shoots are illustrated in Figure 2.

The seedlings, from which the main shoot had been excised, produced new shoots from the growth of axillary buds at the cotyledonary node (Figure 3). These shoots were suitable for further excision 14 to 16 days after the previous shoot had been cut off.

The B_1 seedlings from all the backcrosses and the T_1 seedlings from the testcrosses were tested for their reaction to races 179 and 258 of M. lini by the use of the excised shoot technique.

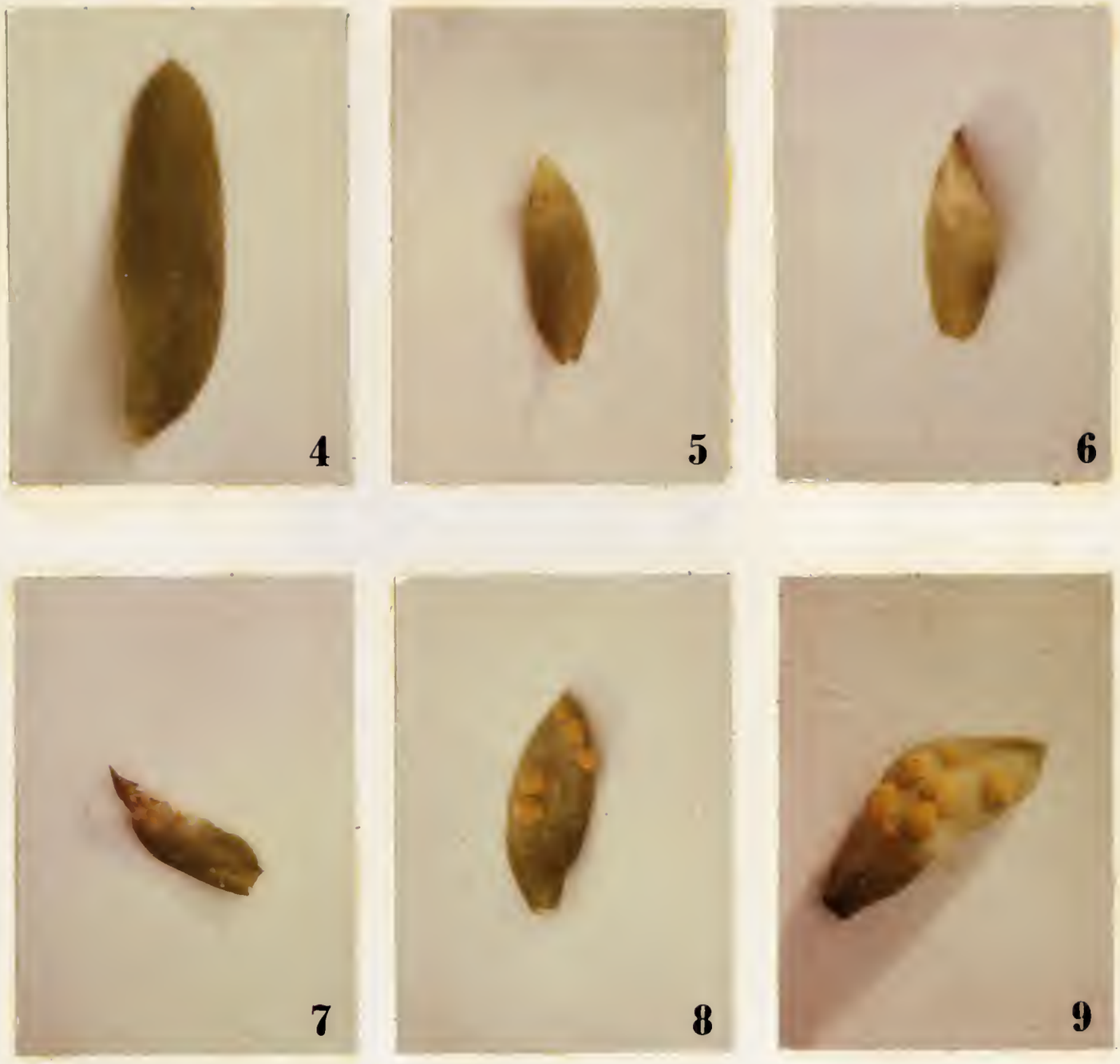
Figure 2. Trays containing excised shoots.

Figure 3. New shoots on seedlings from which a shoot had been excised 14 days previous.

The rust reaction of the inoculated seedlings and excised shoots was classified as either immune or susceptible. The immune reaction was characterized by complete absence of any sign of rust infection on the inoculated leaves and stem of the seedling or shoot

(Figure 4). Non-immune plants showed varying degrees of susceptibility ranging from resistance (Figures 5, 6, 7) to susceptibility (Figures 8, 9). In this study all segregates which showed any degree of infection (Figures 5, 6, 7, 8, 9) were classified as susceptible.

A susceptible and an immune excised shoot are illustrated in Figure 10.



Figures 4-9. Reaction types of race 179 on F_2 segregates of hybrids between Linum usitatissimum and Linum angustifolium.

Figure 4. Immune

Figures 5-9. Susceptible.

Figure 10. Susceptible and immune shoots 14 days after inoculation.

RESULTS AND DISCUSSION

The F_1 hybrids between the rust immune lines of L. angustifolium and the rust susceptible varieties of L. usitatissimum were immune to rust. These hybrids showed the same immunity as their L. angustifolium parents. These results indicated that rust immunity in 8-2 and 13-4 was dominant to susceptibility.

In this study the results from reciprocal crosses were pooled because Chi-square tests for homogeneity showed them not to be different. This finding indicated that immunity to rust was not maternally inherited in either line 8-2 or 13-4.

Genetic Analysis of Rust Immunity in L. angustifolium (8-2)

The classification of the rust reaction of the F_2 progenies from the hybrids, Bison x 8-2 and Redwing x 8-2, with race 258 of M. lini resulted in the number of immune and susceptible F_2 segregates closely approximating a genetic ratio of 3:1 immune to susceptible seedlings (Tables 1 and 2).

TABLE 1. RUST REACTION OF PARENTS, F_1 AND F_2 PROGENIES, OF 8-2 X BISON TO RACE 258; AND GOODNESS OF FIT OF F_2 SEGREGATES TO A 3:1 RATIO.

Hybrid	Reaction to Race 258(2)					Total	χ^2	P value between
	Bison	8-2	F_1	F_2 seedlings				
Bison x 8-2	S	I	I	I	S			0.30
Observed frequencies				157	45			and
Theoretical expectation 3:1				151.5	50.5	202	0.799	0.50

(2) I, immune, S, susceptible.

TABLE 2. RUST REACTION OF PARENTS, F_1 AND F_2 PROGENIES, OF 8-2 X REDWING TO RACE 258; AND GOODNESS OF FIT OF F_2 SEGREGATES TO A 3:1 RATIO.

Hybrid	Reaction to Race 258					Total	χ^2	P value between
	Redwing	8-2	F_1	F_2 seedlings				
Redwing x 8-2	S	I	I	I	S			0.30
Observed frequencies				140	40			and
Theoretical expectation 3:1				135	45	180	0.740	0.50

These data in Tables 1 and 2 indicate that 8-2 carries one dominant gene conditioning immunity to race 258 of M. lini.

The results obtained from testing the T_1 progenies from the testcrosses: (8-2 x Stewart) x Bison, (8-2 x Dakota) x Bison and (8-2 x Akmolinsk) x Bison, with races 179 and 258 are shown in Tables 3, 4 and 5 respectively.

TABLE 3. RUST REACTION OF PARENTS AND T_1 PROGENIES, OF (8-2 X STEWART) X BISON TO RACES 179 AND 258; AND GOODNESS OF FIT OF T_1 SEGREGATES TO A 1:1 RATIO.

Race	Reaction to indicated races of M. lini							Total	χ^2	P value between
	8-2	Stewart	Bison	T_1 seedlings						
179	I	I	S	I	I	I	I			
258	I	S	S	I	I	S	S			0.05
Observed frequencies				171		137				and
Theoretical expectation 1:1				154		154		308	3.76	0.10

None of the T_1 segregates in Table 3 showed susceptibility to race 179. This observation indicated that the gene in 8-2

conditioning immunity to race 179 is allelic to the L gene for rust immunity present in the variety Stewart. This gene was designated as LA by the author to correspond with the allelic nomenclature used by Myers (23) and Flor (12, 14). The number of T_1 seedlings in Table 3 which were immune and susceptible to race 258 did not give a very close fit to the expected 1:1 monogenic backcross ratio (P between 0.10 and 0.05). However, the observed ratio was suggestive of the presence of only one gene conditioning immunity to race 258 in this cross and the fit of the F_2 segregates to a 3:1 monogenic ratio when infected with race 258 (Tables 1 and 2) indicated this to be the case.

TABLE 4. RUST REACTION OF PARENTS AND T_1 PROGENIES, OF (8-2 X DAKOTA) X BISON TO RACES 179 AND 258.

Race	Reaction to indicated races of M. lini			T_1 seedlings				Total
	8-2	Dakota	Bison					
179	I	S	S	I	I	S	S	
258	I	S	S	I	S	I	S	
Observed frequencies				127	11	4	131	273

The classification of the T_1 seedlings in Table 4 indicates that 8-2 contains two genes conditioning immunity to M. lini. One of these genes, previously shown to be allelic to the L gene of Stewart, conditions immunity to race 179 but not to race 258. This gene has been designated L_A. The other gene in 8-2 conditions immunity to race 258 but not to race 179 and was designated as gene X by the author.

These two genes are assumed to be linked because of the wide departure from the expected 1:1:1:1 ratio (Chi-square value due to linkage = 216.28).

TABLE 5. RUST REACTION OF PARENTS AND T_1 PROGENIES, OF (8-2 X AKMOLINSK) X BISON TO RACES 179 AND 258.

Race	Reaction to indicated races of M. lini							Total
	8-2	Akmolinsk	Bison	T_1 seedlings				
179	I	I	S	I	I	S	S	
258	I	I	S	I	S	I	S	
Observed frequencies				174	4	2	81	261

In analyzing the data shown in Table 5, the presence of four distinct phenotypic classes and their wide departure from a 1:1:1:1 backcross ratio supports the hypothesis of two closely linked genes for rust immunity in 8-2. Gene L_A conditions immunity to race 179 but not to race 258 and gene X conditions immunity to race 258 but not to race 179. This testcross, however, involves the variety Akmolinsk which carries gene P_1, conditioning immunity to both races 179 and 258. From the reaction of the T_1 seedlings of (8-2 x Akmolinsk) X Bison to races 179 and 258 it was impossible to separate those segregates which were immune by virtue of the presence of either or both of the closely linked genes L_A and X of 8-2 from those which were immune because of the P_1 gene from Akmolinsk. Flor (12) has shown that the L gene of the variety Stewart is inherited independently of the P_1 gene of the variety

Akmolinsk. It is shown that the L_A gene of 8-2 is allelic to the L gene of Stewart (Table 3) and since genes L_A and X appear to be closely linked it may be assumed that both genes L_A and X are inherited independently of gene P_1 of Akmolinsk.

In the segregation of the T_1 progenies, from the cross (8-2 x Akmolinsk) x Bison, the four parental type phenotypic classes represented by L_AXP_1, L_AX, l_AxP_1 and l_Axp_1 would predominate and the four recombinant phenotypic classes L_AXp_1, L_AxP_1, l_AXP_1 and l_AXp_1 would appear infrequently because of the linkage in the coupling phase of genes L_A and X.

Gene P_1 of Akmolinsk conditions immunity to both races 179 and 258, thus it masks the expression of l_A and x in half of the T_1 segregates. Following the assumption that genes L_A and X are inherited independently of gene P_1 of Akmolinsk it is possible to derive the frequencies of the four phenotypic rust reaction classes due to the segregation of L_A/l_A and X/x independently of gene P_1 (Table 6).

The presence of the P_1 gene masks the expression of one half of the T_1 seedlings with the phenotypes L_Ax and l_AX. To derive the frequency of these phenotypic classes from the observed data the frequency of observed individuals in each of these classes is multiplied by two:

Phenotypic class	Observed frequency	Derived frequency
L_Ax	4	8
l_AX	2	4

This calculation removes six individuals from the 174 which were observed to show immunity to both races 179 and 258. The number of remaining T_1 segregates showing immunity to both these races is thus 174-6 or 168. One third of these 168 individuals would show susceptibility to both races 179 and 258 were it not for the presence of the P_1 gene from Akmolinsk. The derived frequency of the T_1 seedlings with the phenotype $l_A x$ is thus: $\frac{168}{3} + 81 = 137$. The remaining two thirds of the T_1 segregates showing immunity to both races represents the frequency of the phenotypic class $L_A X$, $\frac{2}{3} \times 168 = 112$.

TABLE 6. OBSERVED AND DERIVED FREQUENCIES OF THE T_1 PHENOTYPIC RUST REACTION CLASSES, OF (8-2 X AKMOLINSK) X BISON, IDENTIFIED BY THEIR REACTIONS TO RACES 179 AND 258.

Race	Reaction to indicated races of M. lini											Total
	Genes for immunity			T_1 seedlings								
	8-2		Akmolinsk	Phenotypic classes								
	L_A	X	P_1	$L_A X P_1$	$L_A X p_1$	$l_A x P_1$	$l_A x p_1$	$L_A x p_1$	$L_A x P_1$	$l_A X P_1$	$l_A X p_1$	
179	I	S	I	I	I	I	S	I	I	I	S	
258	S	I	I	I	I	I	S	S	I	I	I	
Observed frequencies					174		81	4			2	
Derived classes				112		137		8		4		261

Strong evidence for linkage between genes L_A and X is obtained from the derived frequencies of the phenotypic classes in Table 6 (Chi-square value due to linkage = 232.39).

The crossover distance between gene L_A and gene X was calculated from combining the observed data of the testcross

(8-2 x Dakota) x Bison and the derived data of the testcross (8-2 x Akmolinsk) x Bison (Tables 4 and 6). This distance was found to be 5.1 ± 1.0 crossover units.

Genetic Analysis of Rust Immunity in L. angustifolium (13-4)

The classification of F_2 progenies from the cross between Bison and 13-4 for their reaction to race 179 is reported in Table 7.

TABLE 7. RUST REACTION OF PARENTS, F_1 AND F_2 PROGENIES, OF 13-4 X BISON TO RACE 179; AND GOODNESS OF FIT OF F_2 SEGREGATES TO A 13:3 RATIO

Hybrid	Reaction to race 179					Total	χ^2	P value between
	13-4	Bison	F_1	F_2 seedlings				
Bison x 13-4	I	S	I	I	S			0.10
Observed frequencies				540	108			and
Theoretical expectation 13:3				526.5	121.5	648	1.85	0.20

The classification of the F_2 progenies of the hybrids Bison x 13-4 and Redwing x 13-4 for their reaction to race 258, is reported in Tables 8 and 9 respectively. These results as well as those in Table 7 show a fit to a 13:3 ratio of immune to susceptible F_2 seedlings.

TABLE 8. RUST REACTION OF PARENTS, F_1 AND F_2 PROGENIES, OF 13-4 X BISON TO RACE 258; AND GOODNESS OF FIT OF F_2 SEGREGATES TO A 13:3 RATIO

Hybrid	Reaction to race 258					Total	χ^2	P value between
	Bison	13-4	F_1	F_2 seedlings				
Bison x 13-4	S	I	I	I	S			0.50
Observed frequencies				159	41			and
Theoretical expectation 13:3				162.5	37.5	200	0.402	0.95

TABLE 9. RUST REACTION OF PARENTS, F_1 AND F_2 PROGENIES, OF 13-4 X REDWING TO RACE 258, AND GOODNESS OF FIT OF F_2 SEGREGATES TO A 13:3 RATIO

Hybrid	Reaction to race 258					Total	χ^2	P value between
	Redwing	13-4	F_1	F_2 seedlings				
Redwing x 13-4	S	I	I	I	S			0.50
Observed frequencies				160	35			and
Theoretical expectation 13:3				158.4	36.6	195	0.082	0.95

The classification of the rust reaction of the B_1 segregates from the backcross (13-4 x Bison) x Bison is shown in Table 10. These B_1 segregates were tested for their reactions to race 179 and 258 by the excised shoot technique. It is shown in Table 10 that the number of immune and susceptible B_1 seedlings fit a 1:1 ratio.

TABLE 10. RUST REACTION OF PARENTS AND B_1 PROGENIES, OF (13-4 X BISON) X BISON TO RACES 179 AND 258; AND GOODNESS OF FIT OF B_1 SEGREGATES TO A 1:1 RATIO

Hybrid	Race	Rust reaction to indicated races of M. lini				Total	χ^2	P value between
		Bison	13-4	B_1 seedlings				
	179	S	I	I	S			
(Bison x 13-4) x Bison	258	S	I	I	S			0.30
Observed frequencies				91	81			and
Theoretical expectation 1:1				86	86	172	0.581	0.50

The classification of the rust reactions of the F_2 hybrids shown in Tables 7, 8 and 9 indicate that 13-4 contains two genes which condition immunity to races 179 and 258 of M. lini. The fit

of the number of immune and susceptible F_2 segregates to a 13:3 ratio suggests that one of these genes is completely dominant, conditioning immunity in the homozygous or heterozygous condition; while the other gene must be present in two doses to condition immunity. This second gene appears to be incompletely dominant in its expression, and plants heterozygous for this gene alone would exhibit a susceptible reaction.

The results in Table 10 indicate that 13-4 contains only one gene which is capable of conditioning immunity while in the heterozygous state. This is indicated by the fit of the ratio of immune to susceptible B_1 segregates to a 1:1 mono-genic backcross ratio.

Further tests of rust reaction should be carried out on F_3 progenies of Bison x 13-4 to conclusively prove this hypothetical gene action in 13-4.

A F_2 ratio of 13:3 immune to susceptible plants from a hybrid between immune L. angustifolium and susceptible L. usitatissimum was found in the data of Deshpande and Jeswani (5). These authors attempted to fit their data to a 3:1 immune to susceptible mono-genic F_2 ratio but were unable to do so. A reanalysis of their data by the author indicated a good fit to a 13:3 ratio (P between 0.30 and 0.50).

CONCLUSION

Two lines of L. angustifolium, 8-2 and 13-4, showed immunity to races 179 and 258 of M. lini. This rust immunity was found to be dominantly inherited in both lines. The results of reciprocal crosses revealed that there was no maternally inherited influence upon rust immunity in either of these lines.

Line 8-2 of L. angustifolium was found to carry two dominant genes conditioning immunity to rust. One of these genes belonged to the L allelic series of rust immunity conditioning genes in L. usitatissimum and conditioned immunity to race 179 but not to race 258. This gene was designated by the symbol L_A. The other gene found in line 8-2 conditioned immunity to race 258 but not to race 179. This gene was designated by the symbol X. These two genes were found to be linked with a distance of 5.1 ± 1.0 crossover units between them.

Line 13-4 of L. angustifolium was also found to carry two genes conditioning immunity to rust. One of these genes was completely dominant and conditioned immunity to races 179 and 258 while in either the homozygous or heterozygous condition. The other gene found in 13-4 also conditioned immunity to both races 179 and 258 but was only able to do so when in the homozygous condition. Its effect in conditioning immunity to rust appeared to be that of incomplete dominance and two doses were necessary for the immune reaction to be expressed.

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